Spectrophotometric Determination of Nicotine

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A rapid and accurate method is presented for determination of nicotine by ultraviolet spectrophotometry. The value of the method is illustrated by comparing analyses of a wide variety of tobacco and nicotine samples by this method and the gravimetric silicotungstic acid method. Neither method differentiates between nicotine and nornicotine.

DURING a study seeking richer natural sources of nicotine and improved methods for its recovery from tobacco, a rapid reliable method was required for determination of nicotine in samples of many different types.

The silicotungstic acid method of the Association of Official Agricultural Chemists, based on a procedure proposed by Chapin (*4*) as a modification of Bertrand's (*2*) method, yielded accurate results for most samples. The method, however, is extremely slow; it requires 24 to 48 hours. Moreover, as shown by Ogg *et al.* (*9*), it is subject to serious errors when large amounts of ammonia or ammonium salts are present. Another defect, usually not serious, is that it does not distinguish nornicotine from nicotine.

The methods of Kissling (*7*), Toth (*12*), and Garner (*5*) are based on the titration of nicotine after isolation of the free base by distillation, extraction, or a combination of these procedures. Because these methods measure all basic material in the test solution as nicotine, they lead to erroneous results in the presence of other bases, which are difficult to separate from nicotine. The colorimetric method proposed by Markwood (*8*), depending on formation of the nicotine 2-naphthylamine–cyanogen bromide complex, is relatively rapid but is not specific for nicotine, because it is subject to interferences by a large number of other substances (*13*). The dipicrate method (*10*) is subject to possible error because of the solubility of the picrate salt.

Other investigators have attempted to shorten the time for analysis of nicotine in tobacco and similar materials by employing micro and semimicro distillation techniques (*1*, *6*). These methods, however, magnify sampling errors, and difficulties caused by frothing are encountered in using the Avens apparatus.

BASIS FOR ULTRAVIOLET ABSORPTION METHOD

The work of Swain *et al.* (*11*) on the ultraviolet absorption of nicotine and related compounds suggested that direct ultraviolet spectrophotometry might be applicable to the quantitative determination of nicotine. The ultraviolet absorption spectrum of nicotine is characterized by a moderately strong, sharp absorption maximum near 260 mμ. The exact position and intensity of the maximum are influenced by the nature of the solvent, and the intensity is markedly affected by acid (Figure 1). Thus the specific extinction coefficient and wave length of the absorption maximum are 18.6 at 260 mμ in water, and 34.3 at 259 mμ in acidified water. (The value 34.3 is an average of five separate weights of a sample of purified nicotine.) Because sensitivity of detection is greater in the latter medium, direct ultraviolet spectrophotometry is admirably suited to determination of nicotine in the acidified aqueous media in which it is commonly obtained or isolated in the laboratory. The applicability of the method to the determination of nicotine in a variety of test solutions was therefore investigated.

The acid concentration is not critical, provided it exceeds 0.02 *N*. Solutions used in this investigation were acidified with hydrochloric acid to 0.05 *N*. Acids such as sulfuric or phosphoric are equally suitable. Spectral densities of solutions, measured on a Beckman Model DU spectrophotometer, were confined to the range 0.2 to 0.8 by adjustment of concentration, or in extremely dilute solutions by using cell lengths up to 5 cm. The acidified nicotine solutions obeyed Beer's law over the range tested, 1 to 30 mg. per liter.

Interferences to be expected in this method are of two types: structurally related alkaloids containing the pyridine chromophore, and unidentified constituents absorbing in this region of the spectrum, either of which may accompany nicotine in the process used for its isolation.

Nornicotine, if present, will be determined as nicotine, because it has the same chromophore and a spectrum indistinguishable from that of nicotine (*11*). The error is somewhat enhanced by the fact that the specific extinction coefficient of nornicotine is

10% higher than that of nicotine. Other methods for determination of nicotine are subject to the same limitation. For most samples, however, and especially those from which the nicotine has been separated by steam distillation, the positive error contributed by nornicotine is usually unimportant, as nornicotine is present in most tobaccos only in small proportion.

Interferences of the second type may include substances such as alkaloid degradation products, plant pigments, and lipides. Extraneous absorbing constituents are more abundant in nicotine solutions obtained by extraction than by distillation procedures. The absorption spectra of a number of typical solutions obtained by both procedures were examined. By comparing these spectra with that of pure nicotine, it was found that the extraneous "background" absorption underlying the nicotine absorption in the spectra of the sample was essentially linear from 230 to 290 mμ. It therefore became a simple matter to correct for this extraneous absorption.

The background correction (*3*) as applied here involves measurement of the spectral densities at 259 mμ (maximum) and at wave lengths 23 mμ on each side of the maximum—that is, at 236 mμ (near minimum) and 282 mμ. For pure nicotine in acidified water, the relationship between the observed densities at these wave lengths is $D_{259} = 1.059\ [D_{259} - {}^1/_2(D_{236} + D_{282})]$. If a solution contains, in addition to nicotine, extraneous substances having linear absorption characteristics (regardless of slope) between 236 and 282 mμ, it can be shown that $D'_{259} = 1.059\ [D_{259} - {}^1/_2(D_{236} + D_{282})]$, where D_{259}, D_{236}, and D_{282} are the observed densities and D'_{259} is the density corrected for background absorption—that is, the density contributed by the nicotine in the solution. The difference between observed and corrected densities, $D_{259} - D'_{259}$, is a measure of the extraneous absorption. The magnitude of corrections in the wide variety of samples studied here ranged from 0 to 5%. However, this correction is valid only when the background absorption is linear or nearly linear from 236 to 282 mμ.

PROCEDURE

An aqueous solution containing the alkaloid and having an acid concentration equivalent to approximately 0.05 *N* hydrochloric acid is ordinarily prepared from a source such as the following: a commercial or laboratory preparation of nicotine or nicotine concentrate; an acidified water extract of a solution of nicotine in solvents such as kerosene and gasoline; or a steam distillate, collected in dilute acid, of a material containing nicotine. The acidified solution, representing a known sample weight, is made to volume and diluted with acidified water, if necessary, until spectral densities observed at wave lengths 236 and 259 mμ lie within the optimum range 0.2 to 0.8. Acidified distilled water is used as a blank for the density measurements. Dilutions are simplified if a sample containing 10 to 20 mg. of nicotine is used and the original volume is made to 1 liter. The observed density in a 1-cm. cell at 236 mμ will then be in the specified range. The density at 282 mμ need not be measured with high accuracy and can therefore be determined on this same solution. The density at the maximum, 259 mμ, is about 10 times that at 236 mμ, and in general will require a tenfold dilution of the solution in order to obtain a value within the specified range.

The density D'_{259}, corrected for background absorption and referred to the original volume, V, which was used in the determination of D_{236}, is calculated by the equation

$$D'_{259} = 1.059\ [FD_{259} - {}^1/_2(D_{236} + D_{282})]$$

where D_{236} and D_{282} are the densities observed at 236 and 282 mμ in V, and D_{259} is the observed density in the diluted solution (dilution factor F, usually 10).

The concentration of nicotine in V, in grams per liter, is then

$$c = D'_{259}/34.3\ b$$

where 34.3 is the specific extinction coefficient (defined by $k = D/bc$), or spectral density referred to cell depth of 1 cm. and concentration of 1 gram per liter of pure nicotine in acidified water at 259 mμ; and b is the inside depth of the cell in centimeters. The depth of the cell is usually 1 cm., but depths up to 5 cm. are used for analyses of solutions low in nicotine.

The total weight of nicotine in the sample, in grams, is simply the concentration, c, times the original volume, V, which is usually 1 liter.

The spectrophotometric method permits the estimation of nicotine in an acidified aqueous solution in 10 to 15 minutes, depending on the number of dilutions necessary.

COMPARISON OF SPECTROPHOTOMETRIC AND A.O.A.C. METHODS

To compare the reliability, precision, and accuracy of the spectrophotometric method for nicotine with that of the official A.O.A.C. silicotungstate method, ten successive analyses of each of two solutions containing different concentrations of nicotine were made by each method (Tables I and II). The nicotine concentrations of the two solutions were near the limits specified by the A.O.A.C. method. For the solution of high nicotine concentration, which contained approximately 100 mg. of nicotine per 100 ml., the standard deviation of the results of the spectrophotometric analyses was 0.144, as compared with 0.089 for the results

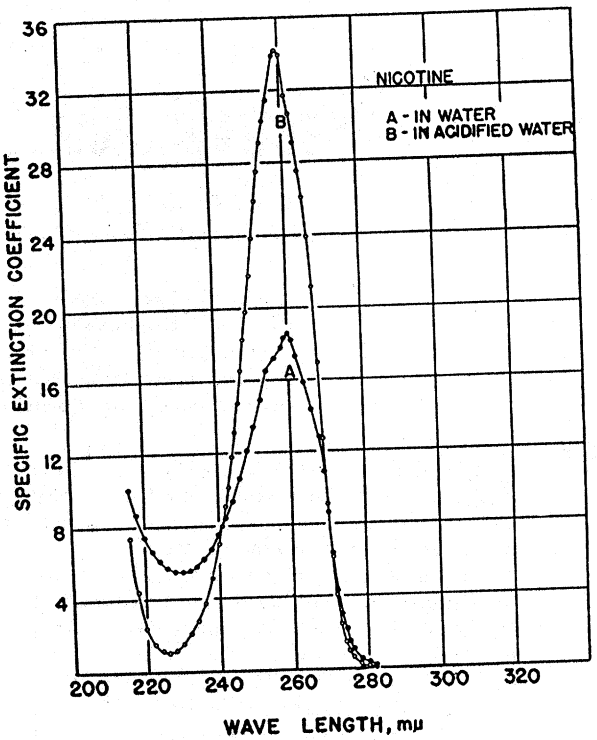

Figure 1. Ultraviolet Absorption Spectrum of Nicotine

A. In water
B. In acidified water, 0.05 *N* HCl

Table I. Determination of Nicotine in Solutions High in Nicotine

Nicotine Found, Mg. per 100 Ml.	
Spectrophotometric	Chemical
102.8	101.3
102.8	101.5
102.8	101.3
102.8	101.3
103.0	101.3
102.5	101.2
102.5	101.2
102.7	101.4
102.8	101.2
102.8	101.3
$\bar{X}$ 102.75	$\bar{X}'$ 101.30
S 0.144	S 0.089

F observed = 2.58
10% level, F = 3.18
2% level, F = 5.35

Table II. Determination of Nicotine in Solutions Low in Nicotine

Nicotine Found, Mg. per 100 Ml.	
Spectrophotometric	Chemical
10.57	10.30
10.60	10.22
10.56	10.24
10.60	10.08
10.56	10.14
10.60	10.18
10.55	10.26
10.57	10.29
10.62	10.27
10.61	
$\overline{X}$ 10.584	$\overline{X}'$ 10.220
S 0.021	S 0.071

F observed = 11.0
2% level F = 5.91

obtained by the A.O.A.C. chemical method. The F value limit indicates that both methods are equally reliable. For the solution of low nicotine content, which contained approximately 10 mg. of nicotine per 100 ml., the results of the analyses show a standard deviation of 0.021 mg. for the spectrophotometric method and 0.071 mg. for the chemical method, indicating a greater precision for the spectrophotometric method. The observed F value of 11.0 when compared with 5.91, the F value for the 2% level, indicates greater precision for the spectrophotometric method for solutions low in nicotine.

The effects of ammonium and alkali salts on the analysis of nicotine by the spectrophotometric method were investigated, because the work of Ogg *et al.* (*9*) had demonstrated that the silicotungstic acid method is subject to considerable error when these salts are present, especially when the nicotine concentration is low. The two basic nicotine solutions used for these tests contained approximately 10 and 80 mg. of nicotine per 100 ml. of solution, representing the approximate limits of nicotine concentration recommended for analysis by the A.O.A.C. method. Nicotine-salt solutions were prepared by adding 10% ammonium chloride, ammonium sulfate, and sodium sulfate, respectively, to the two basic nicotine solutions. High salt concentrations were chosen to exaggerate the effect.

Table III. Effects of Ammonium and Sodium Salts on Determination of Nicotine in Solutions

Salt	Concentration %	Low Nicotine: Spectrophotometric	Low Nicotine: Chemical	High Nicotine: Spectrophotometric	High Nicotine: Chemical
			Mg./100 Ml.		
None	0	10.67	10.59 10.53	82.2	81.3
Ammonium chloride	10	10.61	7.29 10.41	81.9	91.7 94.0
Ammonium sulfate	10	10.61	6.44 6.27	82.1	88.1 85.7
Sodium sulfate	10	10.61	8.22 6.27	82.4	81.3 81.2

Table IV. Distillation of Nicotine from Modified Griffith Apparatus

Time, Min.	Slow Distillation Rate: Volume of distillate, ml.	Slow Distillation Rate: Nicotine found, %	Slow Distillation Rate: % of total nicotine	Rapid Distillation Rate: Volume of distillate, ml.	Rapid Distillation Rate: Nicotine found, %	Rapid Distillation Rate: % of total nicotine
Still Charged with Dried Leaf Tobacco Ground to Pass 100-Mesh						
0	0.0	0.0	0.0	0	0.0	0.0
2	0.1	0.0	0.0	..	..	...
4	56.0	8.01	94.9	..	..	...
6	96.2	8.12	96.3	184	8.16	96.7
8	145.4	8.15	96.5	344	8.27	98.0
10	202.4	8.21	97.3	420	8.27	98.0
12	242.2	8.28	98.1	489	8.28	98.1
14	...	...	...	558	8.34	98.9
16	472.3	8.32	98.6	751	8.35	98.9
20	569.3	8.35	98.9	...	...	...
30	854	8.40	99.6	1410	8.38	99.3
60	1852	8.40	99.6	2918	8.46	100.2

Still Charged with Aqueous Nicotine Solution		
	Mg./100 Ml.	
0	0	0
10	410	10.53
24	805	10.59
Original undistilled solution		10.58

Table V. Determination of Nicotine in Comminuted Green Tobacco Leaves

Distillation Apparatus	Nicotine Found, %: Spectrophotometric	Nicotine Found, %: Chemical
	%	%
Modified Griffith	8.08	8.16
	8.13	8.15
	8.21	8.21
	$\overline{X}$ 8.14	8.17
A.O.A.C.	8.13	8.23
	8.11	8.16
	8.13	8.16
	$\overline{X}$ 8.12	8.18

Table VI. Determination of Nicotine in Dried Tobacco Leaves

Sample	Nicotine Found: Spectrophotometric %	Spectrophotometric Av. %	Chemical %	Chemical Av. %
1-a	0.85	0.85	0.85	0.85
1-b	0.85		0.84	
2-a	2.20	2.20	2.21	2.21
3-a	2.46	2.46	2.48	2.48
4-a	3.17	3.17	3.22	3.22
5-a	4.04	4.07	4.08	4.12
5-b	4.09		4.16	
6-a	5.24	5.28	5.35	5.40
6-b	5.32		5.45	
7-a	6.25	6.26	6.44	6.39
7-b	6.26		6.33	
8-a	8.10	8.10	8.05	8.05
8-b	8.09		8.05	

Table III shows the results of the analyses of these solutions by the spectrophotometric and chemical methods. It is apparent from these data that the chemical method yields erroneous results when applied to solutions containing salts of the type shown (*9*). The results are low and inconsistent for the solutions of low nicotine content in the presence of salts. They are high and inconsistent for the solutions of high nicotine content in the presence of salts, except for the solution containing the sodium salt. On the other hand, the spectrophotometric method yields results in excellent agreement for all cases, showing no effect from the dissolved salts.

APPLICATION OF METHOD

The spectrophotometric method has been applied to analyses of a wide variety of tobacco materials, including tobacco leaves, tobacco juice, distillates, tobacco juice extracts, and tobacco ensilage made of the whole plant. All samples were simultaneously analyzed by the official A.O.A.C. silicotungstic acid method.

The aqueous solution of the nicotine from each of these materials was prepared by steam distillation from solutions made strongly alkaline with either barium or sodium hydroxide. The still used was a modification of that of Griffith and Jeffrey (*6*). A test for completeness of distillation of the nicotine by a qualitative silicotungstic turbidimetric method indicated apparent complete recovery after 4 to 6 minutes, whereas the spectrophotometric method showed an actual recovery of only 95 to 96%. A 30-minute distillation time was adopted for the regular procedure. This provides adequate time to complete the spectrophotometric analysis and to operate two stills simultaneously.

A test of the rate of distillation of nicotine from an aqueous solution (in Table IV) showed that complete recovery was attained in less than 10 minutes. The longer time required for the distillation of nicotine from dried plant tissue must therefore be due to the time required for nicotine to diffuse through the tissue cells.

Nicotine was determined in green tobacco leaves by the spectrophotometric and silicotungstic acid methods with nicotine distillates obtained from them with both the modified Griffith and A.O.A.C. steam distillation apparatus. Green leaves were chosen for this comparison because they are difficult to sample and to analyze. A laboratory sample was prepared by comminuting the leaves in a Waring Blendor with acidified water. Aliquots were removed for the nicotine analyses and for moisture determination. The results (Table V) indicate no significant difference between the two methods of distillation or methods of analysis. Table VI shows the excellent agreement obtained between the results of the spectrophotometric and chemical methods on samples of dried tobacco leaves ranging in nicotine content from less than 1 to more than 8%.

Table VII shows the results of analyses of tobacco juice, distillates, residues, kerosene extracts, and extract residues, and distillates of ensilaged whole tobacco plants. In all cases there was excellent agreement between the spectrophotometric and chemical methods.

Table VII. Determination of Nicotine in Tobacco Products

Material	Nicotine Found, %	
	Spectrophotometric method	Chemical method
	%	%
Tobacco juice	0.52	0.52
Distillate from tobacco juice	0.59	0.58
Residue in still pot	0.20	0.18
Distillate containing ammonia	0.18	0.17
Distillate from potassium chloride solution	0.58	0.58
Juice stripped with kerosene	0.20	0.20
Kerosene extract	0.34	0.34
Whole plants		
Ensilage (with phosphoric acid)	0.50	0.51
Ensilage (with lime)	0.40	0.38
Ensilage (with sodium chloride)	0.32	0.32
Ensilage (with potassium chloride)	0.37	0.36

SUMMARY

A rapid, sensitive method of nicotine analysis is proposed which gives results concordant with the official A.O.A.C. silicotungstic acid method. Nornicotine, if present, is determined as nicotine by both methods. The spectrophotometric method is unaffected by ammonia or alkali salts in the test solution.

When the method is used in conjunction with a modified Griffith and Jeffrey nicotine distillation apparatus, the time required to determine nicotine by the spectrophotometric method is only 20 to 30 minutes, as compared with 24 to 48 hours required by the A.O.A.C. silicotungstic acid method.

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